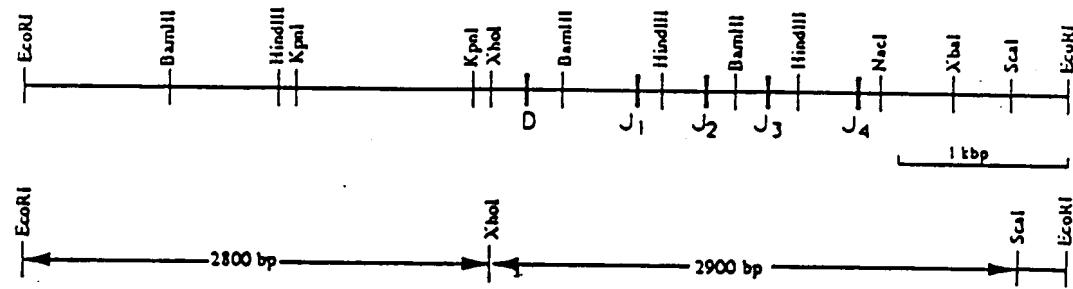


Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



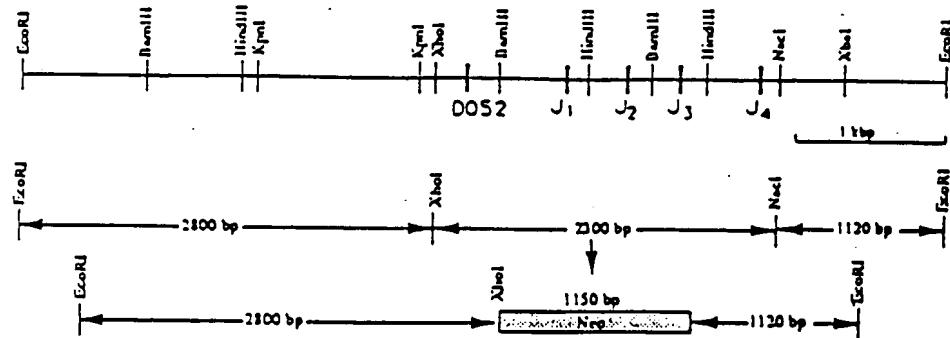
(B) Inactivation vector mDAJ.Neo



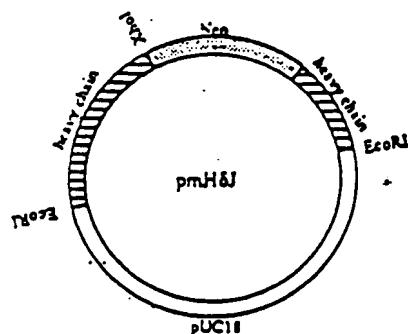
Figure 1

E1124 99007205

(A) Targeted mouse heavy chain J genes

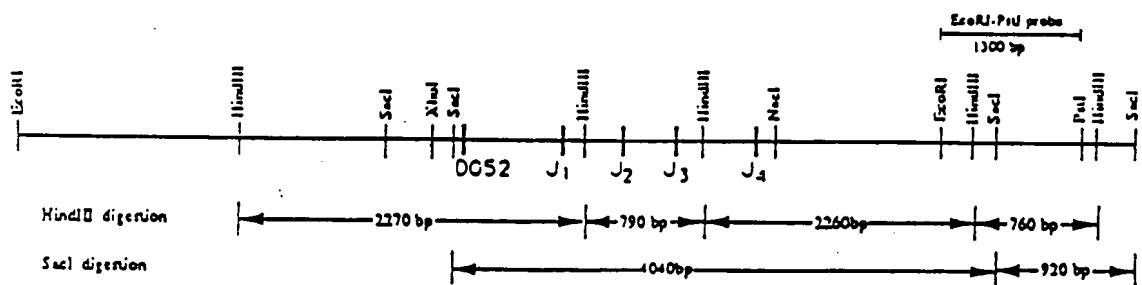


(B) Inactivation vector pmH5J



(C) Southern analysis of pmH δ J-targeted ES colonies

Wild type ES cell genome



Targeted ES cell genome

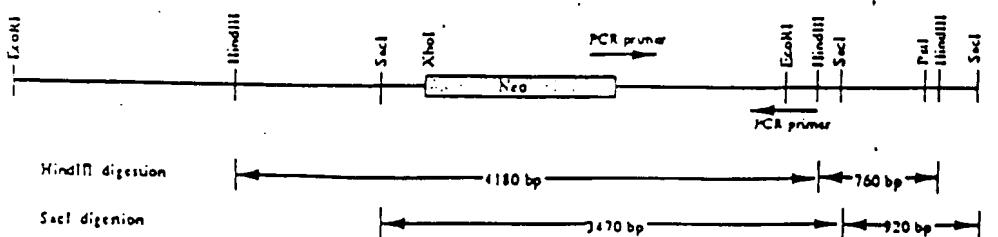
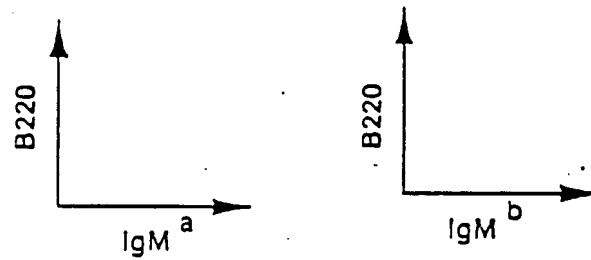
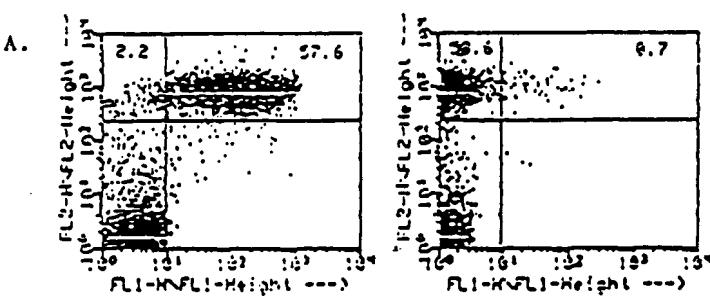


Figure 2

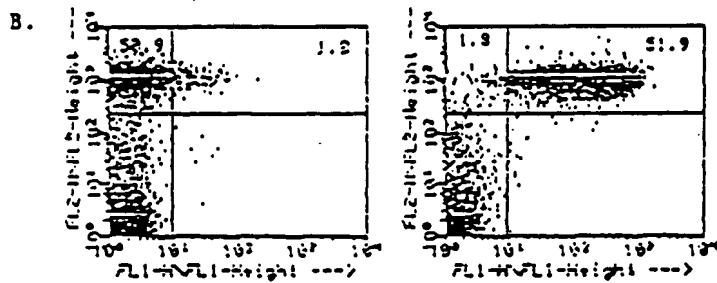
J_H deletion blocks cell surface IgM expression



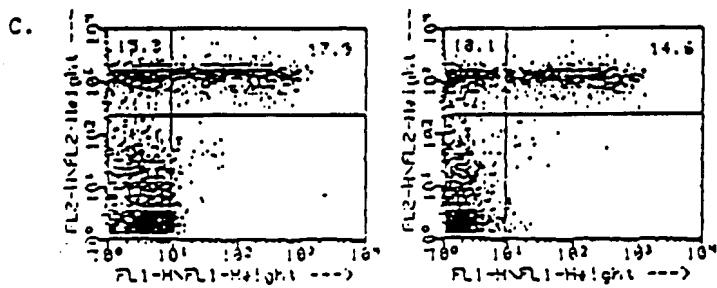
a allotype



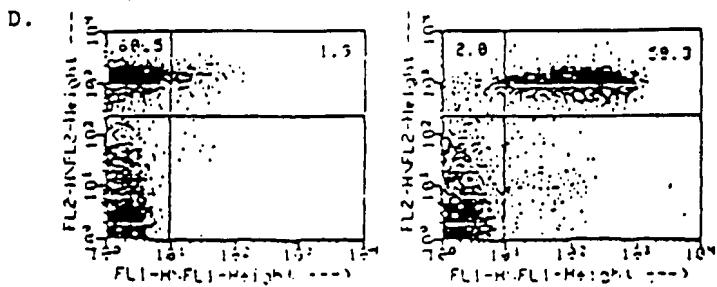
b allotype



a / b F1



ΔJ_H / b F1



Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B, E) or anti-B220 (C, F). (A, B, C) JH-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control (F) control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

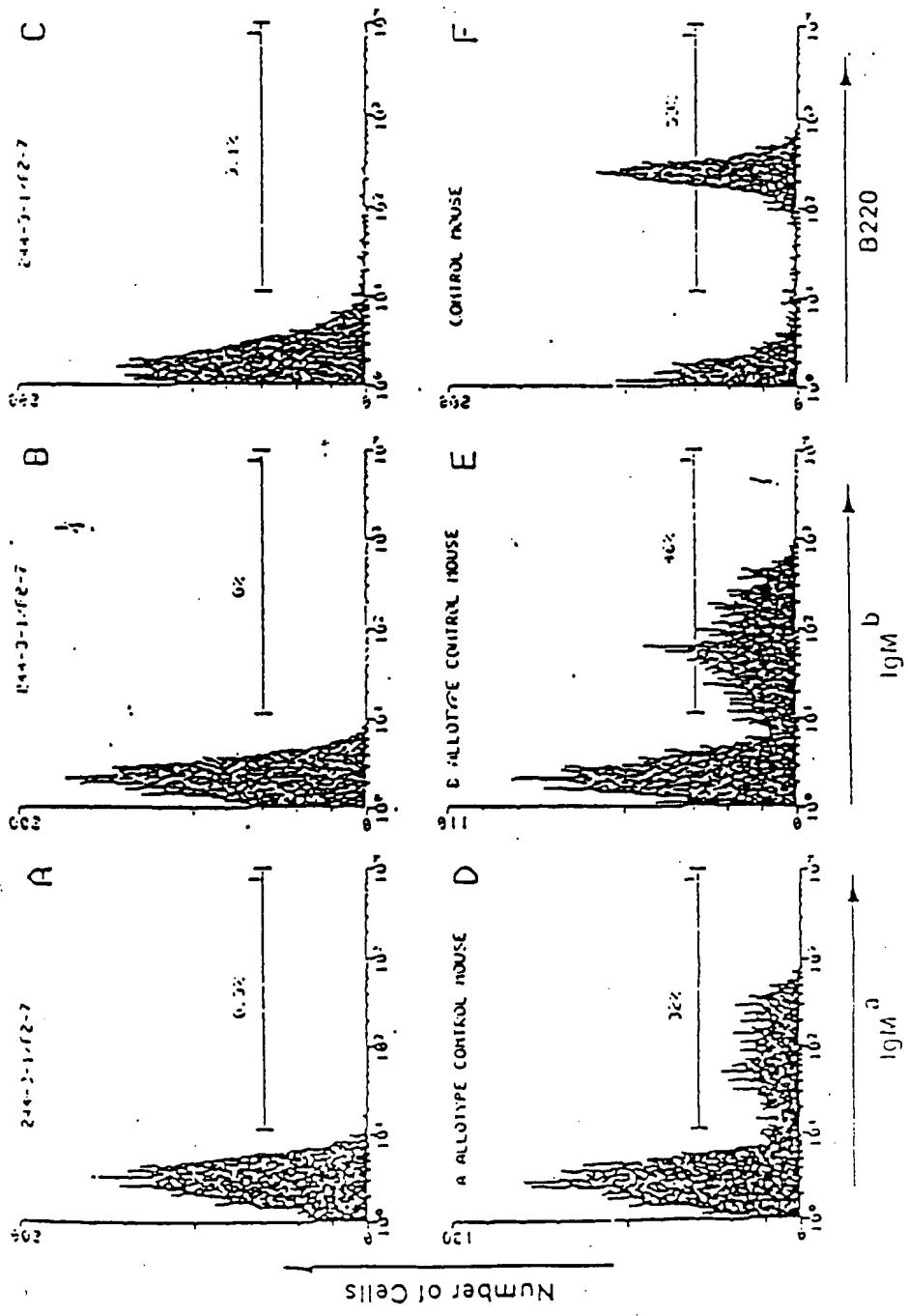


Figure 4

INACTIVATION OF KAPPA CONSTANT REGION

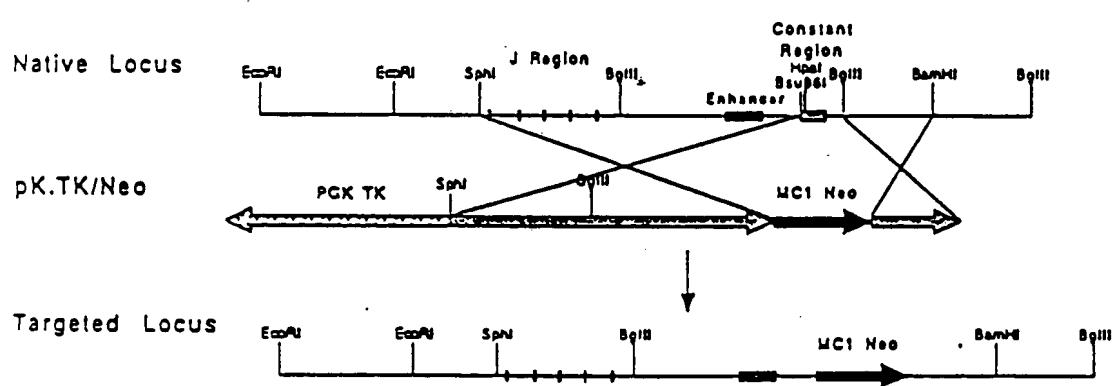


Figure 5

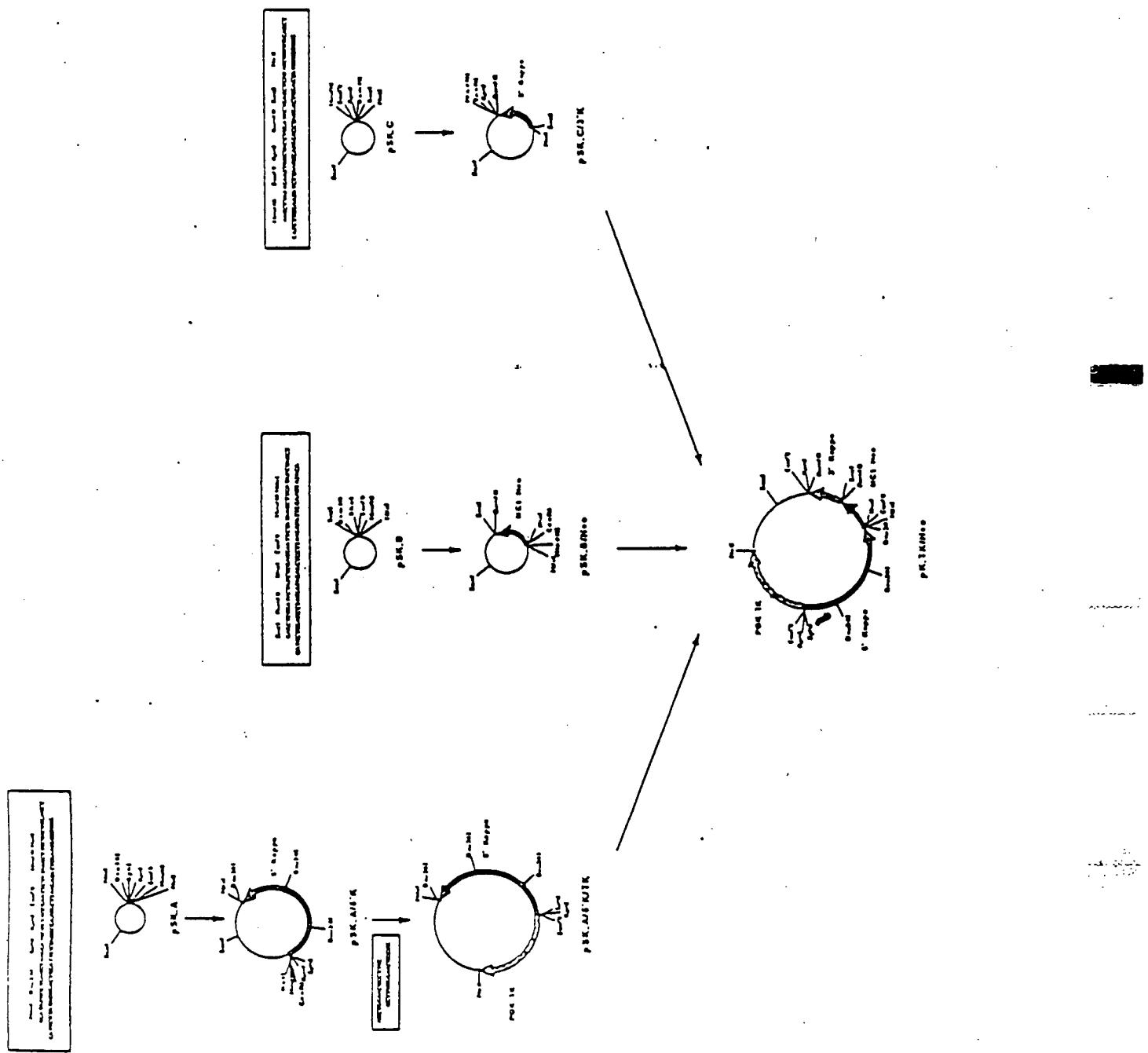
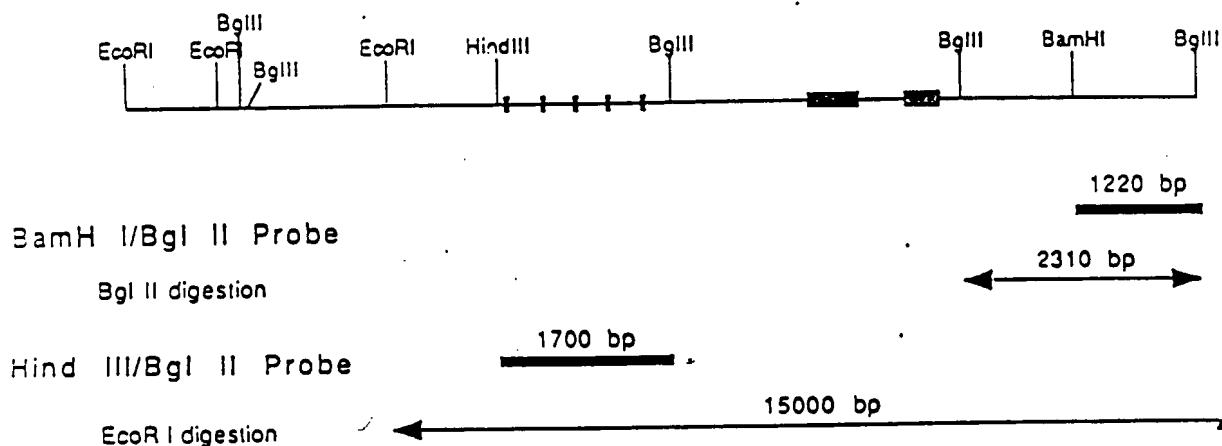


Figure 6

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SOUTHERN ANALYSIS OF LIGHT CHAIN C κ -TARGETED E14-1 CELLS

NATIVE ES CELL LOCUS



TARGETED ES CELL LOCUS

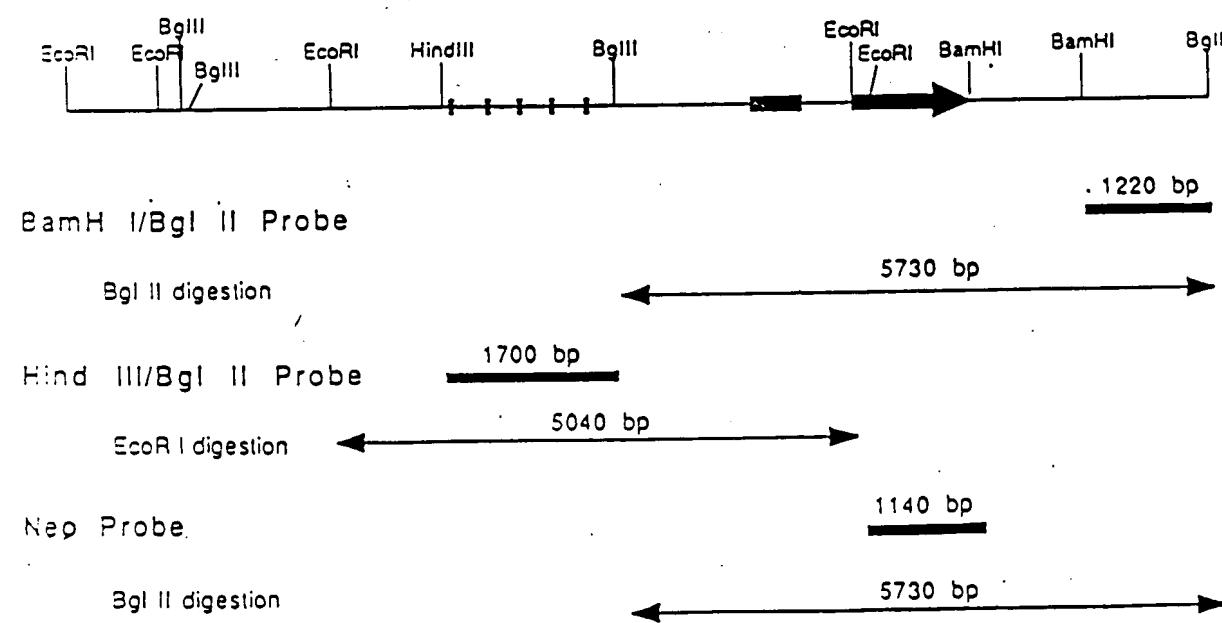
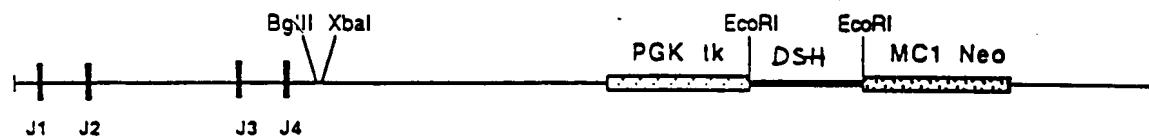


Figure 7

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KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

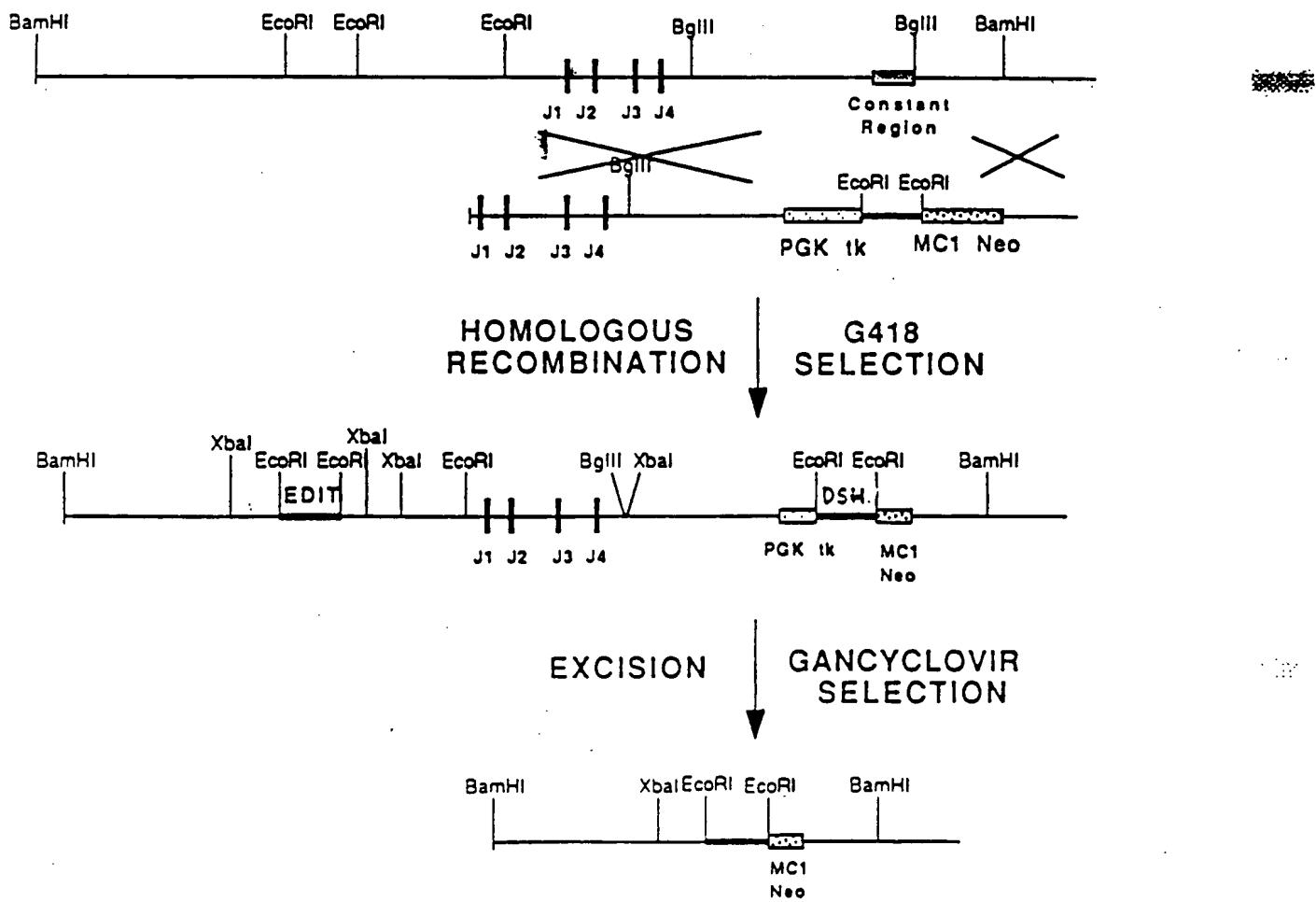


Figure 8

CONSTRUCTION OF KAPPA J/CONSTANT REGION DELETION VECTORS

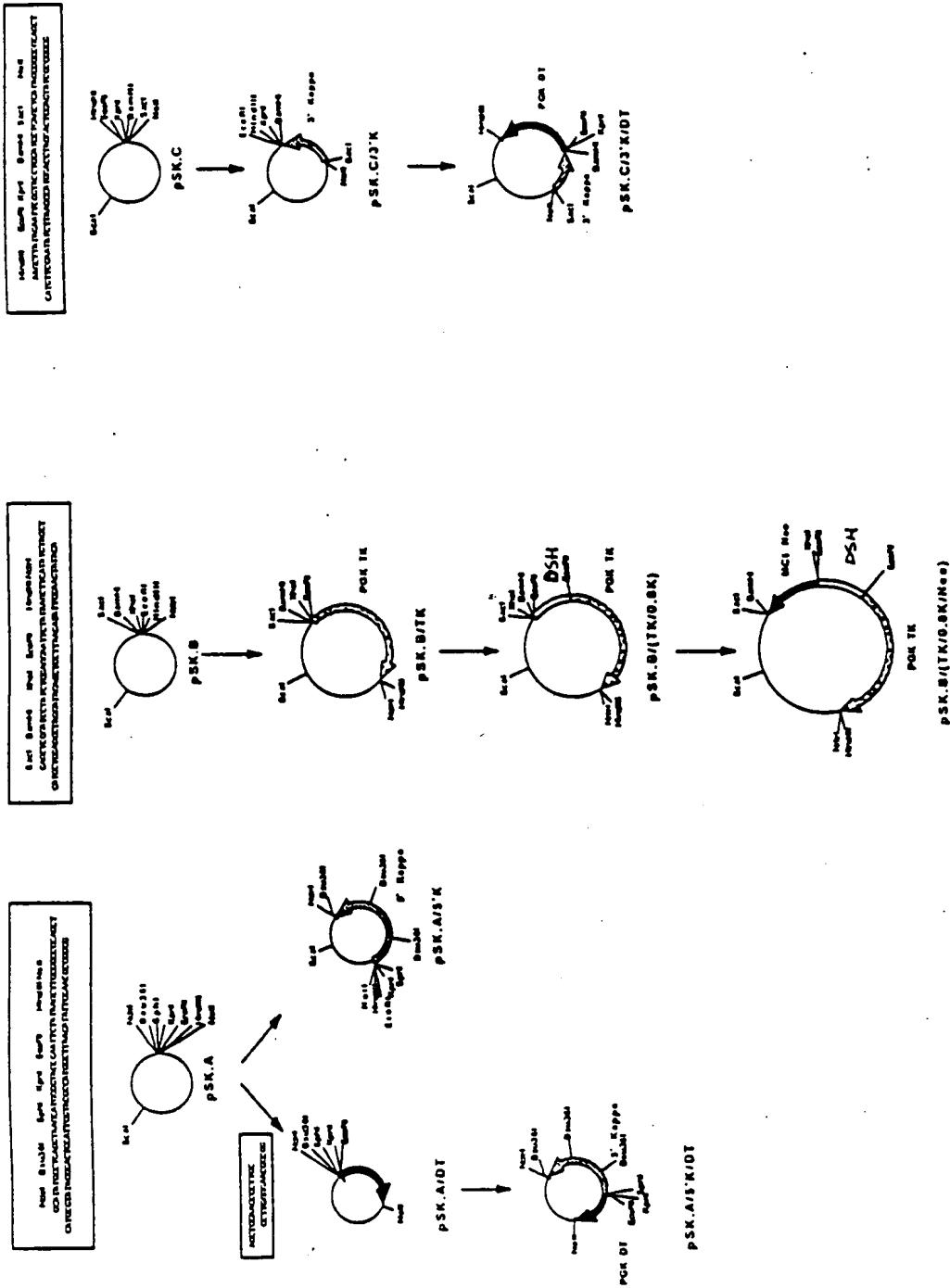


Figure 9

KAPPA J/CONSTANT REGION DELETION VECTORS

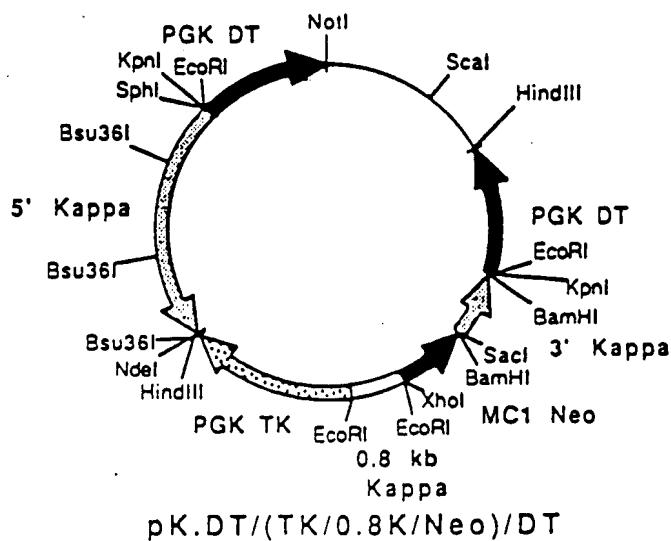
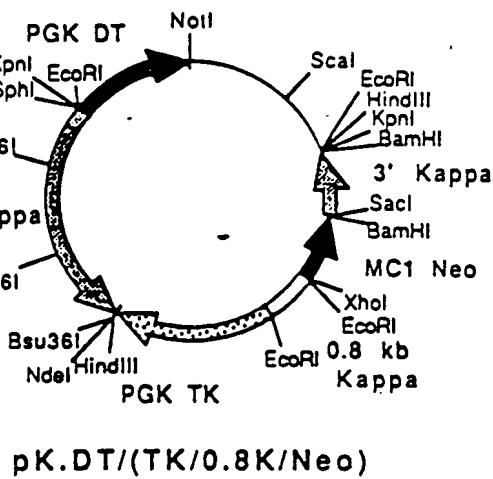
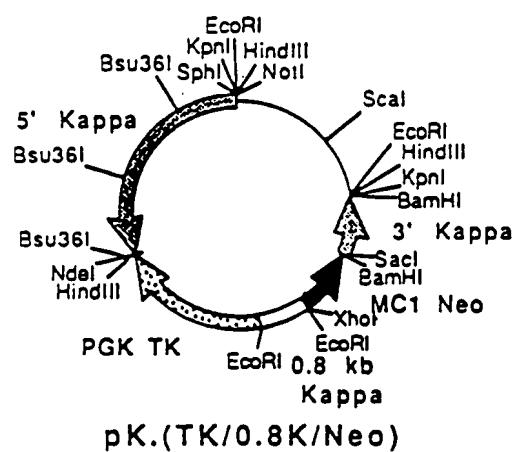
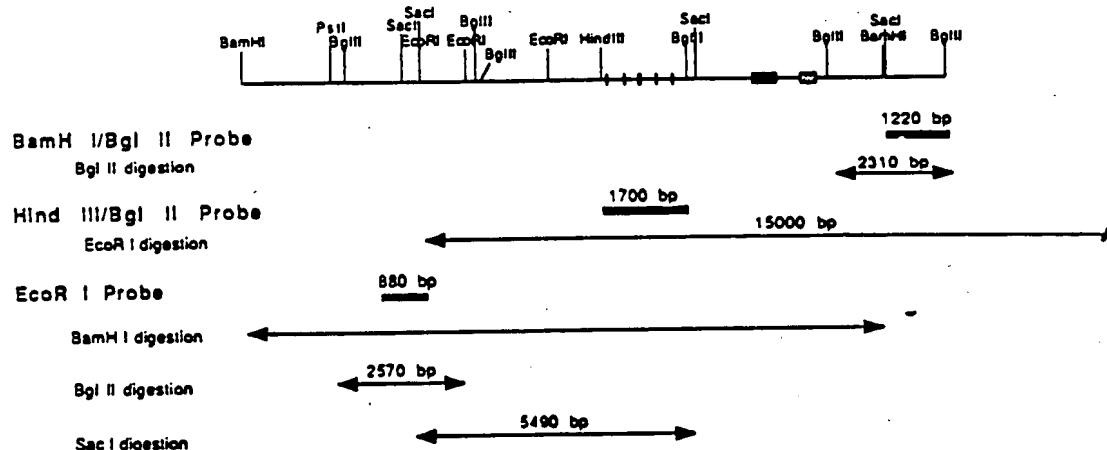


Figure 10

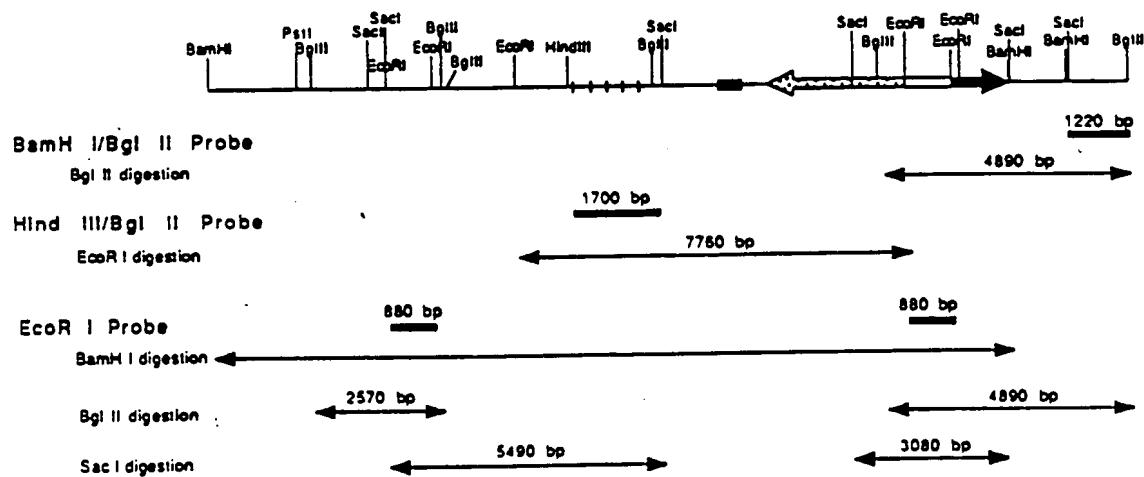
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SOUTHERN ANALYSIS OF LIGHT CHAIN $J\kappa/C\kappa$ -DELETED E14-1 CELLS

NATIVE ES CELL LOCUS



$C\kappa$ -TARGETED ES CELL LOCUS



$J\kappa C\kappa$ -DELETED ES CELL LOCUS

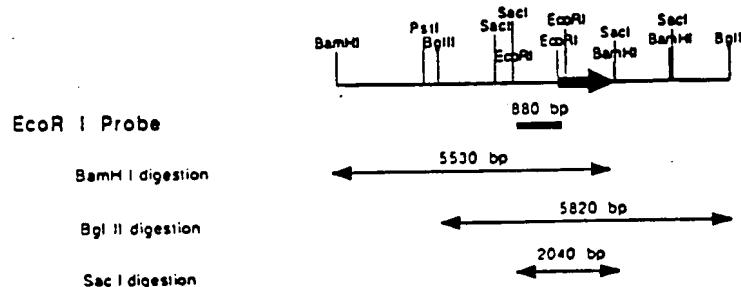


Figure 11

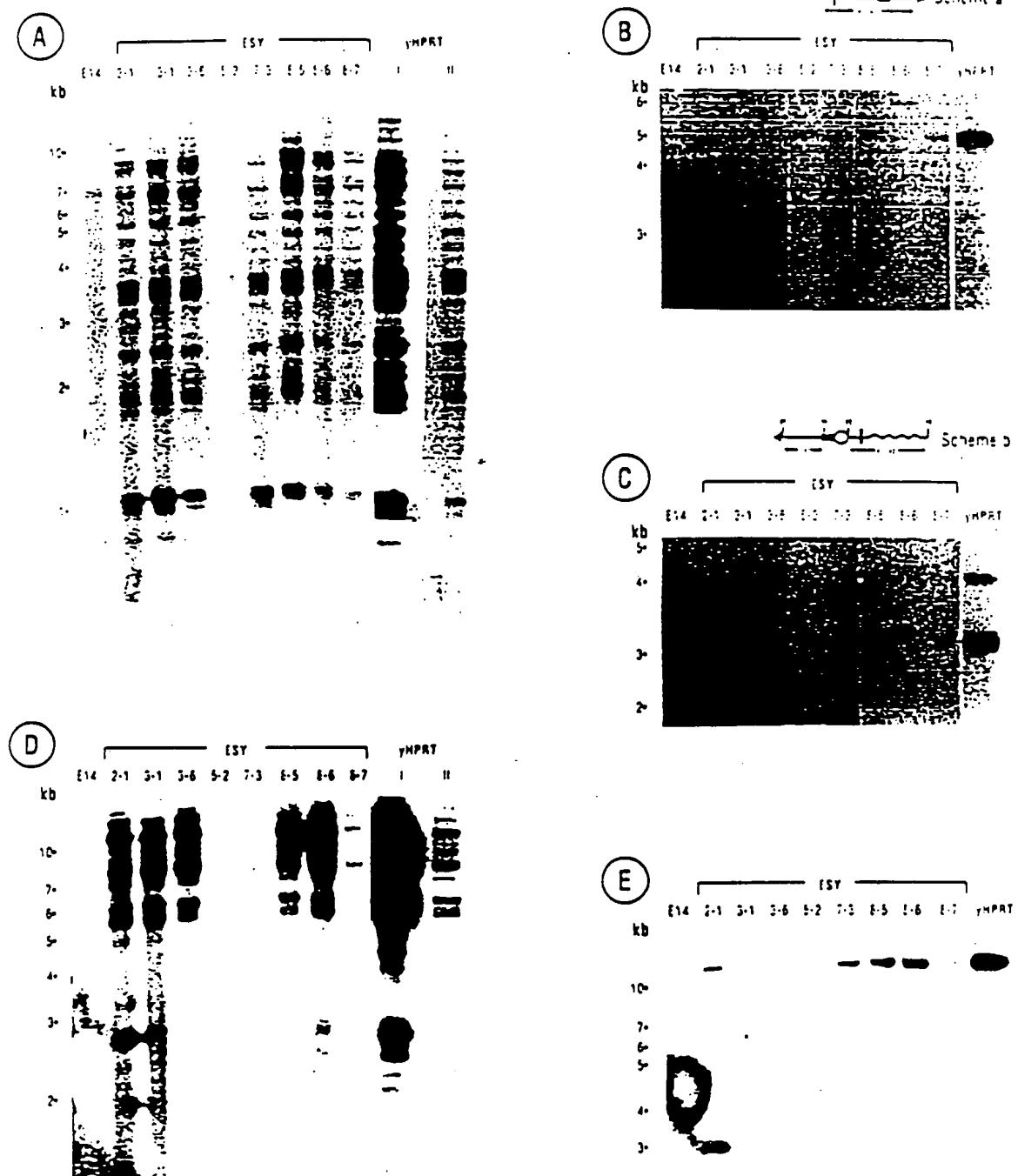


Figure 12

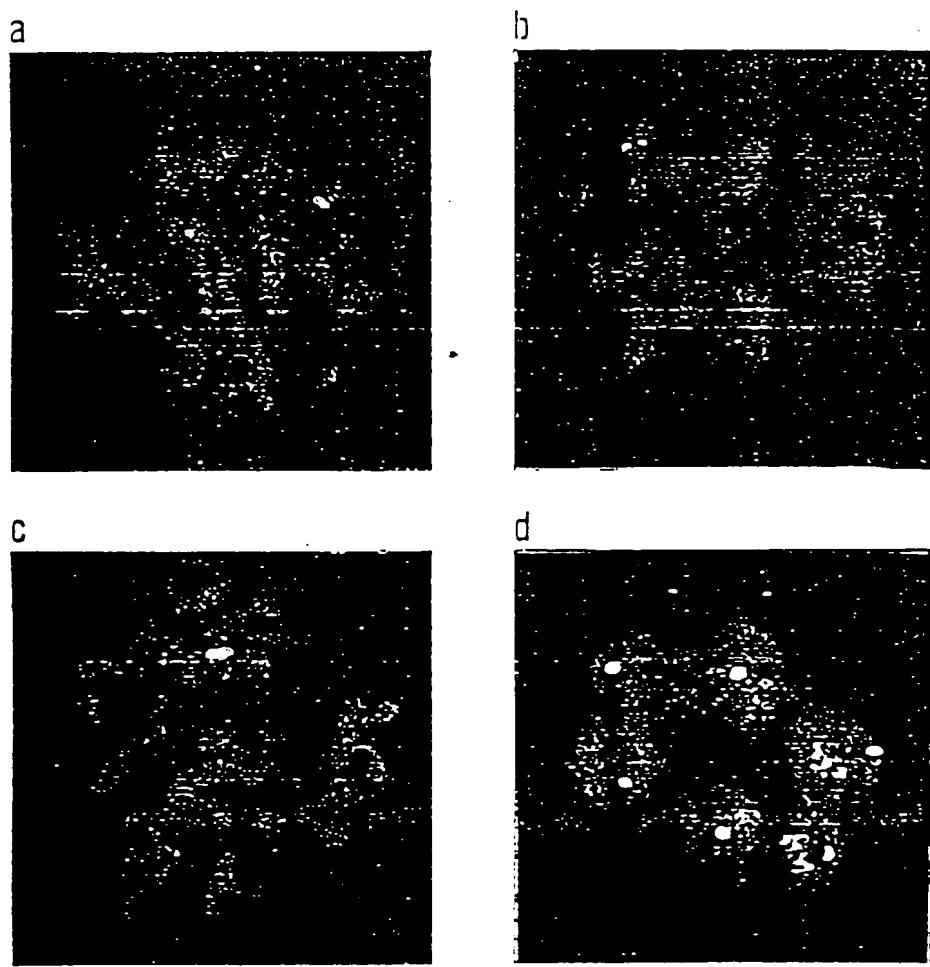
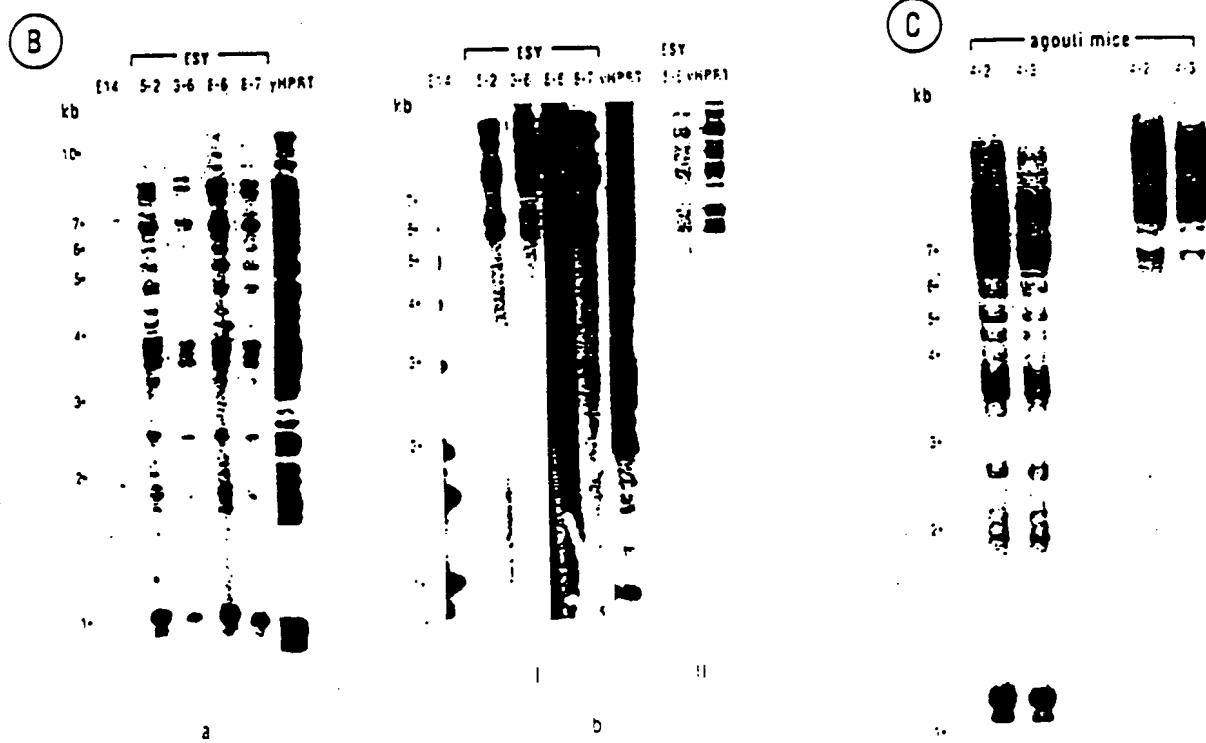
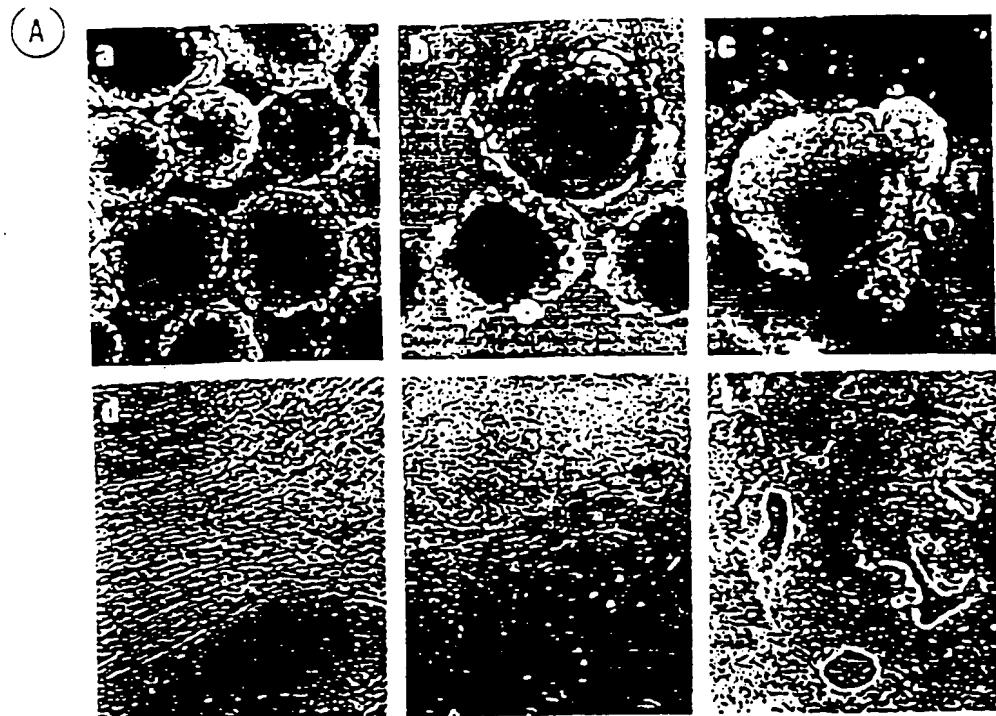


Figure 13



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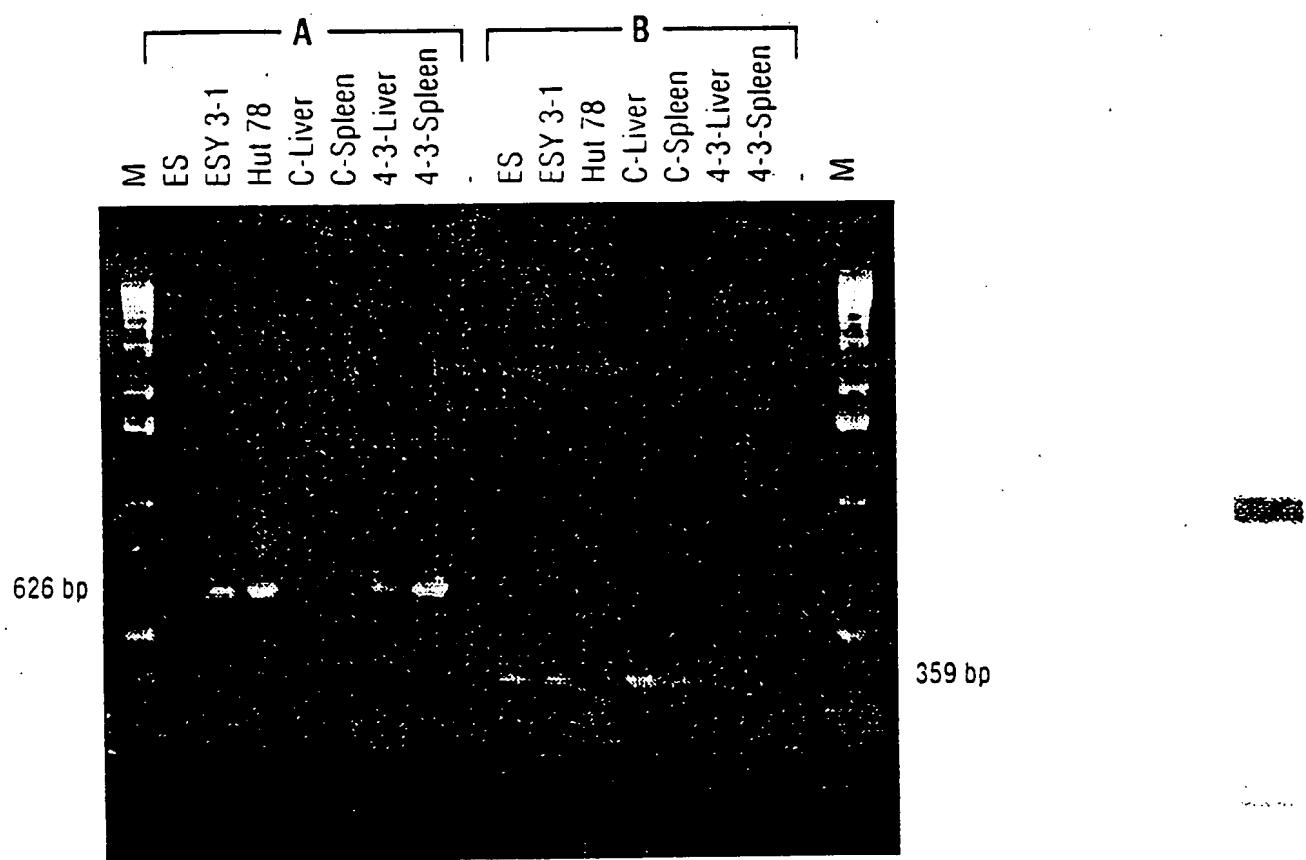
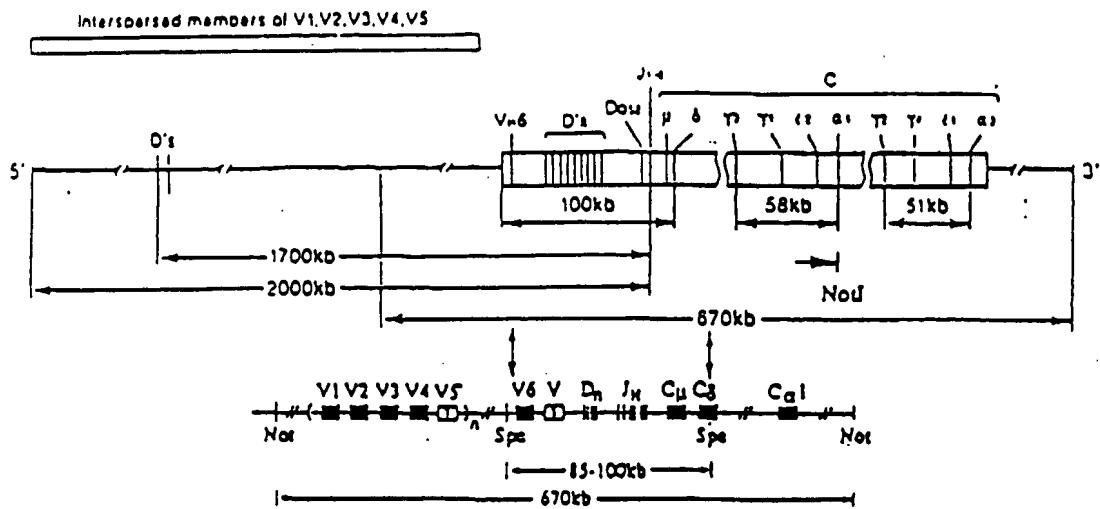
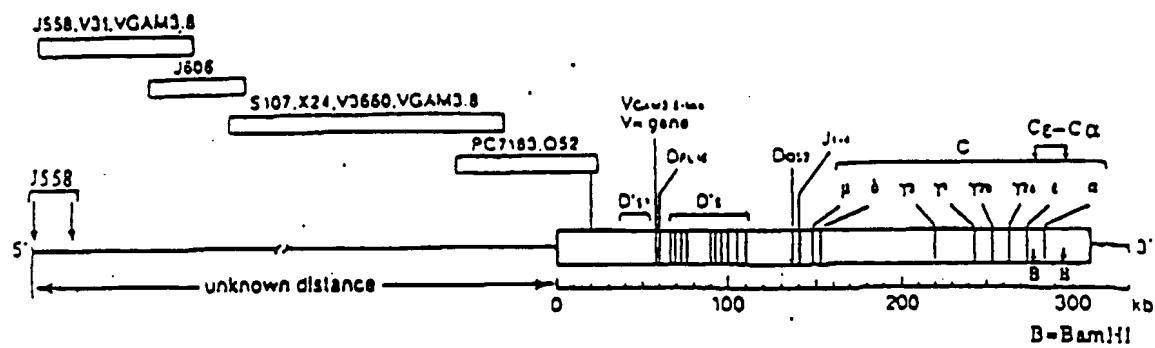


Figure 15

(A) Human heavy chain locus



(B) Mouse heavy chain locus



(C) Human heavy chain replacement YAC vector

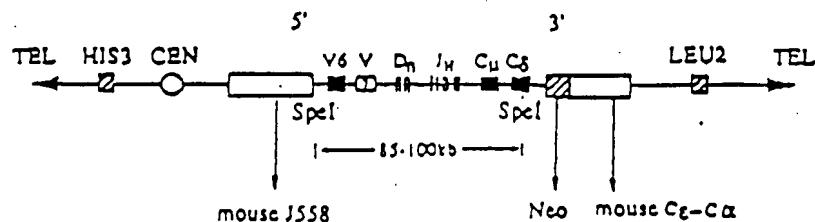


Figure 16

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Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK
MIgH (inactive) MIgK
MIgH MIgK
X
MIgH MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X
heterozygous Human IgK
MIgH MIgK HIgH
MIgH MIgK
X
MIgH MIgK HIgK
MIgH MIgK

↓

↓

F1 (cross I A)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II.

F1 (cross I A) x F1 (cross I B)

↓

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III.

Intercross F2 mice

↓

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

MAMMALIAN HOST GENOTYPES

<u>Hetero- or Hemi-zygous Mice</u>	<u>Intercross Product Mice*</u>
I. <u>Δmi_L mi_H</u> mi _L mi _H	<u>Δmi_L mi_H</u> <u>Δmi_L mi_H</u>
II. <u>mi_L Δmi_H</u> mi _L mi _H	<u>mi_L Δmi_H</u> <u>mi_L Δmi_H</u>
III. <u>mi_L mi_H hi_H</u> mi _L mi _H	<u>mi_L mi_H hi_H</u> <u>mi_L mi_H hi_H</u>
IV. <u>mi_L mi_H hi_L</u> mi _L mi _H	<u>mi_L mi_H hi_L</u> <u>mi_L mi_H hi_L</u>
V. Animal I X Animal II	
<u>Δmi_L mi_H</u> mi _L Δ mi _H	<u>Δmi_L Δmi_H</u> <u>Δmi_L Δmi_H</u>
VI. Animal III X Animal V	
<u>mi_L mi_H hi_H</u> Δ mi _L mi _H	<u>Δmi_L mi_H hi_H</u> and <u>Δmi_L mi_H hi_H</u> <u>Δmi_L mi_H</u> <u>Δmi_L mi_H</u>
VII. Animal IV X Animal V	
<u>mi_L mi_H hi_L</u> Δ mi _L mi _H	<u>Δmi_L mi_H hi_L</u> and <u>Δmi_L mi_H hi_L</u> <u>Δmi_L mi_H hi_L</u> <u>Δmi_L mi_H</u>
VIII. Animal VI X Animal VII	
<u>Δmi_L mi_H hi_L hi_H</u> Δ mi _L mi _H	<u>Δmi_L mi_H hi_L hi_H</u> <u>Δmi_L mi_H hi_L hi_H</u>
	<u>Δmi_L mi_H hi_L hi_H</u> and <u>Δmi_L mi_H hi_L hi_H</u> <u>Δmi_L mi_H hi_L hi_H</u> <u>Δmi_L mi_H</u>
IX. Animal III X Animal IV	
<u>mi_L mi_H hi_L hi_H</u> mi _L mi _H	<u>mi_L mi_H hi_L hi_H</u> <u>mi_L mi_H hi_L hi_H</u>
X. Animal II X Animal IX	
<u>mi_L mi_H hi_L hi_H</u> mi _L mi _H	<u>mi_L mi_H hi_L hi_H</u> and <u>mi_L mi_H hi_L hi_H</u> <u>mi_L mi_H hi_L hi_H</u> <u>mi_L mi_H</u>
XI. Animal I X Animal IX	
<u>Δmi_L mi_H hi_L hi_H</u> mi _L mi _H	<u>Δmi_L mi_H hi_L hi_H</u> and <u>Δmi_L mi_H hi_L hi_H</u> <u>Δmi_L mi_H hi_L hi_H</u> <u>Δmi_L mi_H</u>

*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain